Amendments to the Claims

Please amend Claims 10, 19, 25, 31 and 39. The Claim Listing below will replace all prior versions of the claims in the application:

Claim Listing

1. (Original) A method of purifying hypothalamic inhibitory factor from a sample containing hypothalamic inhibitory factor comprising subjecting the sample to diafiltration, solid phase extraction and immunoaffinity chromatography.

2. (Original) The method of Claim 1 wherein the diafiltration is in tandem to the solid phase extraction.

3. (Original) The method of Claim 2 wherein the immunoaffinity chromatography follows the solid phase extraction.

4. (Original) A method of purifying hypothalamic inhibitory factor from a sample containing hypothalamic inhibitory factor comprising the steps of:
   a) subjecting the sample to diafiltration to produce a diafiltrate of hypothalamic inhibitory factor;
   b) subjecting the diafiltrate of a) to solid phase extraction to produce a first fraction of hypothalamic inhibitory factor;
   c) subjecting the first fraction of b) to immunoaffinity chromatography, wherein an antibody which binds to hypothalamic inhibitory factor or an HIF binding fragment thereof is coupled to an immunoaffinity column, to produce a second fraction of hypothalamic inhibitory factor;
   d) subjecting the second fraction of c) to reverse phase high pressure liquid chromatography to produce a third fraction of hypothalamic inhibitory factor; and
   e) recovering purified hypothalamic inhibitory factor from the third fraction of d).

5. (Original) The method of Claim 4 wherein the diafiltration is continuous diafiltration.

6. (Original) The method of Claim 5 wherein the continuous diafiltration is a tangential diafiltration.

7. (Original) The method of Claim 4 wherein the solid phase extraction is performed using a C18 silica column and an acetonitrile elution.

8. (Original) The method of Claim 4 wherein the sample is homogenized hypothalamus.

9. (Previously presented) The method of Claim 4 wherein the antibody is a monoclonal antibody or a binding fragment thereof having a binding affinity of from about 3×10^{-7} M to about 5×10^{-7} M for hypothalamic inhibitory factor.

10. (Currently amended) The method of Claim 9 wherein the monoclonal antibody or the binding fragment thereof has the binding affinity of monoclonal antibody 26-10, as deposited with ATCC under HB-8120, for hypothalamic inhibitory factor.

11. (Original) The method of Claim 4 wherein the reverse phase high pressure liquid chromatography is performed by use of a C18 silica column and an acetonitrile gradient elution.

12. (Original) The method of Claim 1 wherein the immunoaffinity chromatography of c) is performed using a methanol gradient.

13. (Original) A method of purifying hypothalamic inhibitory factor comprising the steps of:

a) preparing a tissue slurry from a sample comprising hypothalamic inhibitory factor;

b) obtaining a retentate of the sample of a), wherein the retentate comprises hypothalamic inhibitory factor;

c) diafiltering the retentate of b) to produce a diafiltrate of hypothalamic inhibitory factor;

d) chromatographically treating the ultrafiltrate of c) using solid phase extraction;

e) performing immunoaffinity chromatography on the hypothalamic inhibitory factor obtained in d) using an antibody which binds to hypothalamic inhibitory factor or a hypothalamic inhibitory factor antibody binding fragment thereof;

f) performing reverse phase high pressure liquid chromatography on the hypothalamic inhibitory factor obtained in e);

thereby producing purified hypothalamic inhibitory factor.

14. (Original) The method of Claim 13 wherein the diafiltration is continuous diafiltration.

15. (Original) The method of Claim 14 wherein the continuous diafiltration is a tangential diafiltration.

16. (Original) The method of Claim 13 wherein the solid phase extraction of d) is performed using a C18 silica column and an acetonitrile elution.

17. (Original) The method of Claim 13 wherein the sample is homogenized hypothalamus.

18. (Previously presented) The method of Claim 13 wherein the antibody is a monoclonal antibody or a binding fragment thereof having a binding affinity of from about 3×10^{-7} M to about 5×10^{-7} M for hypothalamic inhibitory factor.

19. (Currently amended) The method of Claim 18 wherein the monoclonal antibody or the binding fragment thereof has the binding affinity of monoclonal antibody 26-10, as deposited with ATCC under HB-8120, for hypothalamic inhibitory factor.

20. (Original) The method of Claim 13 wherein the reverse phase high pressure liquid chromatography of f) is performed by use of a C18 silica column and an acetonitrile gradient elution.

21. (Original) A method of purifying hypothalamic inhibitory factor comprising the steps of:

a) performing tangential diafiltration on a sample comprising hypothalamic inhibitory factor to produce a diafiltrate permeate which includes the hypothalamic inhibitory factor;

b) performing solid phase extraction on the diafiltrate permeate of a) to produce a concentrate of hypothalamic inhibitory factor;

c) performing immunoaffinity chromatography on the concentrate of b) using an antibody having affinity for hypothalamic inhibitory factor or an hypothalamic inhibitory factor antibody binding fragment thereof to produce a hypothalamic inhibitory factor-containing fraction; and

d) performing reverse phase high pressure liquid chromatography on the hypothalamic inhibitory factor-containing fraction of c) to produce purified hypothalamic inhibitory factor.

22. (Original) The method of Claim 21 wherein the solid phase extraction of b) is performed using a C18 silica column and an acetonitrile elution.

23. (Original) The method of Claim 21 wherein the sample is homogenized hypothalamus.

24. (Previously presented) The method of Claim 21 wherein the antibody is a monoclonal antibody or a binding fragment thereof having a binding affinity of from about 3×10^{-7} M to about 5×10^{-7} M for hypothalamic inhibitory factor.

25. (Currently amended) The method of Claim 24 wherein the monoclonal antibody the binding fragment thereof has the binding affinity of monoclonal antibody 26-10, as deposited with ATCC uner HB-8120, for hypothalamic inhibitory factor.

26. (Original) The method of Claim 21 wherein the reverse phase high pressure liquid chromatography of d) is performed by use of a C18 silica column and an acetonitrile gradient elution.

27. (Original) A method of purifying hypothalamic inhibitory factor comprising the steps of:

a) blending a tissue comprising hypothalamic inhibitory factor to form a tissue slurry;

b) centrifuging the tissue slurry of a) to produce a retentate comprising the hypothalamic inhibitory factor;

c) tangentially diafiltering the retentate of b) to produce a diafiltrate permeate comprising the hypothalamic inhibitory factor;

d) chromatographically treating the diafiltrate permeate of c) by solid phase extraction employing an acetonitrile elution and a silica packing to produce a first hypothalamic inhibitory factor fraction in acetonitrile;

e) concentrating the first hypothalamic inhibitory factor fraction of d);

f) chromatographically treating the concentrated hypothalamic inhibitory factor of e) by immunoaffinity chromatography employing a glycine elution and a resin packing, wherein an antibody which binds to hypothalamic inhibitory factor or a hypothalamic inhibitory factor antibody binding fragment thereof is coupled to the resin, to produce a fraction of hypothalamic inhibitory factor in glycine;

g) chromatographically treating the hypothalamic inhibitory factor of f) by reverse phase high pressure liquid chromatography employing an acetonitrile gradient elution and a silica packing to produce a second hypothalamic inhibitory factor fraction in acetonitrile;

h) evaporating the acetonitrile from the second hypothalamic inhibitory factor fraction in acetonitrile of g) thereby producing purified hypothalamic inhibitory factor.

28. (Original) The method of Claim 27 wherein the solid phase extraction of d) is performed using a C18 silica column and an acetonitrile elution.

29. (Original) The method of Claim 27 wherein the sample is homogenized hypothalamus.

30. (Previously presented) The method of Claim 27 wherein the antibody is a monoclonal antibody or a binding fragment thereof having a binding affinity of from about 3×10^{-7} M to about 5×10^{-7} M for hypothalamic inhibitory factor.

31. (Currently amended) The method of Claim ~~28~~ 30 wherein the monoclonal antibody or the binding fragment thereof has the binding affinity of monoclonal antibody 26-10, as deposited with ATCC under HB-8120, for hypothalamic inhibitory factor.

32. (Original) The method of Claim 27 wherein the reverse phase high pressure liquid chromatography of g) is performed by use of a C18 silica column and an acetonitrile gradient elution.

33. (Original) A method of purifying hypothalamic inhibitory factor from a sample containing hypothalamic inhibitory factor comprising subjecting the sample to diafiltration and immunoaffinity chromatography.

34. (Original) A method of purifying hypothalamic inhibitory factor from a sample containing hypothalamic inhibitory factor comprising subjecting the sample to tangential diafiltration, solid phase extraction and immunoaffinity chromatography.

35. (Original) The method of Claim 34 wherein the solid phase extraction is performed using a C18 silica column and an acetonitrile elution.

36. (Original) The method of Claim 34 wherein the sample is homogenized hypothalamus.

37. (Original) The method of Claim 34 wherein the immunoaffinity chromatography is performed using an antibody which binds to hypothalamic inhibitory factor or a

hypothalamic inhibitory factor binding fragment thereof which is coupled to an immunoaffinity column.

38. (Previously presented) The method of Claim 37 wherein the antibody is a monoclonal antibody or a binding fragment thereof having a binding affinity of from about 3×10^{-7} M to about 5×10^{-7} M for hypothalamic inhibitory factor.

39. (Currently amended) The method of Claim 38 wherein the monoclonal antibody or the binding fragment thereof has the binding affinity of monoclonal antibody 26-10, as deposited with ATCC under HB-8120, for hypothalamic inhibitory factor.

40. (Original) The method of Claim 34 wherein the immunoaffinity chromatography is performed using a methanol gradient.